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*Published in:*
Food Additives & Contaminants. Part B. Surveillance

*Link to article, DOI:*
10.1080/19393210.2018.1462258

*Publication date:*
2018

*Document Version*
Peer reviewed version

Link back to DTU Orbit

*Citation (APA):*
Jørgensen, K., & Olesen, P. T. (2018). Kainic acid in the seaweed Palmaria palmata (Dulse). *Food Additives & Contaminants. Part B. Surveillance*, *11*(3), 198-200. https://doi.org/10.1080/19393210.2018.1462258

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Food Additives & Contaminants: Part B
Surveillance

ISSN: 1939-3210 (Print) 1939-3229 (Online) Journal homepage: http://www.tandfonline.com/loi/tfab20

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**To cite this article:** Kevin Jørgensen & Pelle Thonning Olesen (2018): Kainic acid in the seaweed Palmaria palmata (Dulse), Food Additives & Contaminants: Part B, DOI: 10.1080/19393210.2018.1462258

**To link to this article:** https://doi.org/10.1080/19393210.2018.1462258

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**Publisher:** Taylor & Francis

**Journal:** *Food Additives & Contaminants: Part B*

**DOI:** 10.1080/19393210.2018.1462258

**Kainic acid in the seaweed *Palmaria palmata* (Dulse)**

Kevin Jørgensen and Pelle Thonning Olesen

*National Food Institute, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark*

**ABSTRACT**

Twenty samples of the seaweed *Palmaria palmata* (dulse) purchased mainly from commercial internet shops on the European market were analysed by a liquid chromatograph coupled with a tandem mass spectrometer (LC-MS/MS) method for the content of kainic acid, a naturally occurring neurotoxic compound in *P. palmata*. Kainic acid levels in the samples ranged widely from trace levels to approximately 560 µg $g^{-1}$ dry weight.

**Keywords**: Palmaria palmata, dulse, seaweed, kainic acid

CONTACT: Kevin Jørgensen, kejo@food.dtu.dk

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## Introduction

*Palmaria palmata* (Linnaeus) F.Weber & D.Mohr, often referred to as dulse or dillisk is a seaweed/macro alga that has been consumed historically in coastal communities of Ireland, France (Brittany), Iceland, Norway, Scotland, USA (Maine) and Canada (Nova Scotia). Today *P. palmata* is sold for human consumption across Europe. It may be harvested from the littoral zones in the northern Atlantic or Pacific oceans or it can be cultivated. For culinary use the seaweed can be used in a number of ways, e.g. like a salad or as a snack (Mouritsen et al. 2013).

Canadian researchers were the first to report the presence of kainic acid (Figure 1) in *P. palmata* (Laycock et al. 1989; Ramsey et al. 1994). The reported concentrations of kainic acid varied from below the limit of detection and up to 4000 µg $g^{-1}$ dry weight (dw). In two "dwarf mutant" strains the concentrations exceeded 10 000 µg $g^{-1}$ dw. They also found that the kainic acid concentration in the apical, central and basal regions of the macro algae was fairly uniform. Despite kainic acid being a well-known neurotoxin at the time, the finding went largely unnoticed. More recently six additional measurements, including samples from America and Europe, have been reported with contents ranging from 0.22 to 130 µg $g^{-1}$ dw (Mouritsen 2013 et al.). Though data is thus still very scarse, the data indicate that kainic acid can be found in *P. palmata* at highly variable concentrations.

Kainic acid (CAS no. 487-79-6) was originally discovered by Japanese scientists in the 1950s, as an anthelmintic constituent of the seaweed *Diginea simplex* (Wulfen) C. Agardh (Higa & Kuniyoshi 2000). Kainic acid was later shown to have a potential for causing excitatory action on neurons (Shinozaki & Konishi 1970), and its neurotoxic potential was firmly established by Olney et al. (1974). Kainic acid has subsequently become a popular model substance for studying neurophysiology (Higa & Kuniyoshi 2000).

Presence of kainic acid in other kinds of seaweed than *P. palmata* has been investigated to some extend. Sato el al. (1996) investigated the content of kainic acid in 46 marine algae species. Kainic acid was detected in only 3 species: *D.*

*simplex* (maximum 2760 µg $g^{-1}$ dw), the seaweed where kainic acid originally was isolated from, *Laurencia papillosa* (maximum 10 µg $g^{-1}$ dw) and *Osmundaria obtusiloba* (C. Agardh) R.E.Norris (*Vidalia obtusiloba*) (maximum 1 µg $g^{-1}$ dw). Impellizzeri et al. (1975) detected kainic acid in *Centroceras clavulatum* (C. Agardh) Montagne (320 µg $g^{-1}$ dw), but it was not detected in 17 other marine algae. Rozas & Freitas (2007) did not find any kainic acid in the neurotoxic seaweed *Dichotomaria marginata* (J.Ellis & Solander) Lamarck (*Galaxaura marginata*).

Therefore the aim of this study was to acquire more information of kainic acid content originating from *P. palmata* in products sold on the European market.

**Materials and Methods**

***Sample collection***

Twenty samples of *P. palmata* products were collected. Fifteen of the samples were bought in commercial internet shops from 2013 to 2015 and analysed as they were received. Five samples were collected in 2012 to 2014 from coastal waters, four from Denmark and one from Norway. These fresh samples were freeze dried for 24 hours before analysis. Ten grams of each sample were homogenised in a blender before analysis. The content of water was determined in all samples by drying at 70°C under vacuum.

***Chemicals and reagents***

Kainic acid monohydrate was obtained from Sigma-Aldrich (St. Louis, MO, USA). A stock standard solution of kainic acid (0.92 mg $mL^{-1}$) was made in acetonitrile/water (1:9, v/v) and kept at 5°C. Methanol and acetonitrile were of LC/MS grade from Rathburn Chemicals Ltd. (Walkerburn, Scotland). Water was purified by a Milli-Q Reference water purification system (Milford, Bedford, USA). Ammonium formate (98.7%) was from VWR Chemicals (Leuven, Belgium) and formic acid (98-100%) was from Merck (Darmstadt, Germany).

***Sample preparation***

One gram of sample was weighed in a 50 mL plastic centrifuge tube and extracted with 8 mL 50% methanol/water by mixing with a Ultra-Turrax (IKA, Staufen, Germany) for 1 minute. The mixture was centrifuged for 5 minutes at 2630 g and the extract was transferred into a 25 mL volumetric flask. The extraction was resumed with 8 mL 100% methanol by the same procedure and a third extraction was done with 8 mL 50% methanol/water, again by the same procedure. The flask was filled to 25 mL with methanol. This extraction procedure was validated by extraction of the remaining sample portion of naturally positive sample pellets by the same procedure, where no more kainic acid was detected in this extra second extraction procedure. The extract was filtered (Sartorius Minisart, 0.45 µm, Sartorius AG, Goettingen, Germany) before injection into the LC-MS/MS.

**LC-MS/MS quantification**

The HPLC system was a HP 1100 system (Agilent Technologies, Wilmington, DE, USA). A 5 µm amide bonded silica (TSK-gel Amide-80) column (250 x 2.0 mm) from Tosoh Bioscience (Grove, CA, USA) was used at 20°C. The mobile phase was 29% water and 71% acetonitrile containing 2.0 mM ammonium formate and 3.6 mM formic acid. The flow was 0.2 mL $min^{-1}$, the injection volume was 5 µl and the retention time for kainic acid was 5.9 minutes. Mass spectrometry was performed using a Micromass Quattro Ultima triple quadrupole mass spectrometer, equipped with MassLynx software for instrument control and data processing (Waters-Micromass, San Diego, California, USA). Electrospray ionization in the positive mode was used. The electrospray capillary was set at 3.5 kV and the cone at 25 V. The ion source block temperature was set at 130°C, the desolvation temperature at 450°C, and the flow rates for nitrogen bath and spray were 500 L $h^{-1}$ and 90 L $h^{-1}$, respectively. Data were acquired in the MRM (multiple reaction monitoring) mode. The pressure of the argon used for collision-induced dissociation was $2 \cdot 10^{-3}$ mBar and the collision energy was 20 eV. The ions monitored were m/z 122, m/z 150 and m/z 168, daughter ions of kainic acid: m/z 214. Similarly, for 1-hydroxykainic acid the ions monitored were m/z 140, m/z 168 and m/z 186, daughter ions of m/z 232. The quantification of kainic acid was based on the most intense daughter ion m/z 122 at the chosen instrument settings. The other ions were used as confirmative ions and the ion ratio should be within ± 30% (relative) of average of calibration standards from same sequence. The quantification was done by five external standards of matrix

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matched kainic acid standards in the range 280-9400 ng $mL^{-1}$. This corresponds to a quantification range from 7 to 235 μg $g^{-1}$ in seaweed. Samples with higher concentrations were diluted before quantification.

## Results and discussion

### *Method quality assurance*

Experiments at a spiking level of 92 μg $g^{-1}$ gave recoveries of 92 ± 15% (n=4). The analytical results were not corrected for recovery. The limit of quantification (LOQ) was 7 μg $g^{-1}$. Three of the positive samples, in the range 120-240 μg $g^{-1}$, were analyzed in duplicate on two different days and the relative standard deviation ranged from 2 to 8%. Based on these data a conservative estimate of the measurement of uncertainty of results is ± 20%, with a coverage factor 2.

### *Analytical results*

The dry matter content of the fifteen samples bought from commercial shops was in the range of 65-91%. The dry matter content in the five freeze dried samples was in the range of 91-95%. The kainic acid concentration in the fifteen commercially sold products and five freeze dried samples is listed in table 1. In table 2 the kainic acid concentration is corrected for the residual moisture content of the samples into μg $g^{-1}$ dw. This study is the most comprehensive analysis of kainic acid in *P. palmata* to date. The data confirm that kainic acid is a naturally occurring compound in both freshly harvested *P. palmata* and in the dried commercial products sold on the European market. The levels ranged from trace levels to 560 μg $g^{-1}$ dw. Since this is still only a small study it is not unlikely that higher concentrations could be found in other *P. palmata* products on the European market. The results could not confirm the findings of Ramsey et al. (1994), who reported concentrations up to 4 000 μg $g^{-1}$ dw in two *P. palmata* strains and even exceeding 10,000 μg $g^{-1}$ dw in two "dwarf mutant" strains. But overall, the results are in good agreement with previous findings, showing that the kainic acid concentrations in *P. palmata* are highly variable. Ramsey et al. (1994) also reported the presence of 1-hydroxykainic acid in *P. palmate*, whereas in this study only trace levels of this compound were found in some samples.

## Conclusions

Our analysis of samples of *P. palmata* from the European market support the previous findings, that the concentration of kainic acid in *P. palmata* is highly variable, from trace levels to several hundred micrograms pr. gram dry weight. However, if the upper range of kainic acid content in *P. palmata* is to be properly elucidated, additional analysis needs to be conducted. A safe intake level of kainic acid remains to be established, but the data shows that it should be possible to cultivate *P. palmata* for human consumption with very low levels of kainic acid, though further research is needed to identify the factors that determine the kainic acid concentration in the seaweed.

## Disclosure statement

The authors declare that there is no conflict of interest.

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**Funding**

This work was financially supported by The Danish Food and Veterinary Administration.

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Table 1. Kainic acid concentration (µg g-1) in commercially sold or freeze dried samples.

| Country | <7[1] | 7-100 | 101-200 | 201-300 | >300 | Max.[2] | Max.[3] |
|---|---|---|---|---|---|---|---|
| Denmark | 3 | 0 | 1 | 0 | 1 | 500 | 560 |
| Norway | 0 | 0 | 0 | 1 | 0 | 230 | 240 |
| Island | 3 | 1 | 0 | 0 | 0 | 13 | 15 |
| Ireland | 2 | 3 | 0 | 1 | 0 | 240 | 270 |
| China | 1 | 0 | 0 | 0 | 0 | <7 | <7 |
| France | 0 | 1 | 0 | 0 | 1 | 430 | 470 |
| Spain | 0 | 1 | 0 | 0 | 0 | 16 | 18 |

[1] all samples below 7 µg $g^{-1}$ (LOQ) contained trace levels of kainic acid.

[2] unadjusted for moisture content.

[3] adjusted to their dry weight.

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Excel Data

| sample_id* | Analyte* | value* | units* | uncertainty |
|---|---|---|---|---|
| 1 | kainic acid | 240 | µg/g dry weight | ± 20% |
| 2 | kainic acid | < 7 | µg/g dry weight | ± 20% |
| 3 | kainic acid | < 7 | µg/g dry weight | ± 20% |
| 4 | kainic acid | 130 | µg/g dry weight | ± 20% |
| 5 | kainic acid | 15 | µg/g dry weight | ± 20% |
| 6 | kainic acid | < 7 | µg/g dry weight | ± 20% |
| 7 | kainic acid | 270 | µg/g dry weight | ± 20% |
| 8 | kainic acid | 63 | µg/g dry weight | ± 20% |
| 9 | kainic acid | 91 | µg/g dry weight | ± 20% |
| 10 | kainic acid | < 7 | µg/g dry weight | ± 20% |
| 11 | kainic acid | < 7 | µg/g dry weight | ± 20% |
| 12 | kainic acid | < 7 | µg/g dry weight | ± 20% |
| 13 | kainic acid | 60 | µg/g dry weight | ± 20% |
| 14 | kainic acid | < 7 | µg/g dry weight | ± 20% |
| 15 | kainic acid | < 7 | µg/g dry weight | ± 20% |
| 16 | kainic acid | 560 | µg/g dry weight | ± 20% |
| 17 | kainic acid | 470 | µg/g dry weight | ± 20% |
| 18 | kainic acid | < 7 | µg/g dry weight | ± 20% |
| 19 | kainic acid | 18 | µg/g dry weight | ± 20% |
| 20 | kainic acid | 31 | µg/g dry weight | ± 20% |

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| sample_id* | Country* | Year* | Matrix* | Species | Common name | Weight | Length | age | comments |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Norway | 2012 | Palmaria palmata | | dulse | | | | |
| 2 | Denmark | 2012 | Palmaria palmata | | dulse | | | | |
| 3 | Denmark | 2012 | Palmaria palmata | | dulse | | | | |
| 4 | Denmark | 2014 | Palmaria palmata | | dulse | | | | |
| 5 | Island | 2013 | Palmaria palmata | | dulse | | | | |
| 6 | Denmark | 2014 | Palmaria palmata | | dulse | | | | |
| 7 | Ireland | 2014 | Palmaria palmata | | dulse | | | | |
| 8 | Ireland | 2014 | Palmaria palmata | | dulse | | | | |
| 9 | Ireland | 2014 | Palmaria palmata | | dulse | | | | |
| 10 | China | 2014 | Palmaria palmata | | dulse | | | | |
| 11 | Island | 2014 | Palmaria palmata | | dulse | | | | |
| 12 | Island | 2015 | Palmaria palmata | | dulse | | | | |
| 13 | Ireland | 2015 | Palmaria palmata | | dulse | | | | |
| 14 | Island | 2015 | Palmaria palmata | | dulse | | | | |
| 15 | Ireland | 2015 | Palmaria palmata | | dulse | | | | |
| 16 | Denmark | 2015 | Palmaria palmata | | dulse | | | | |
| 17 | France | 2015 | Palmaria palmata | | dulse | | | | |
| 18 | Ireland | 2015 | Palmaria palmata | | dulse | | | | |
| 19 | Spain | 2015 | Palmaria palmata | | dulse | | | | |
| 20 | France | 2015 | Palmaria palmata | | dulse | | | | |

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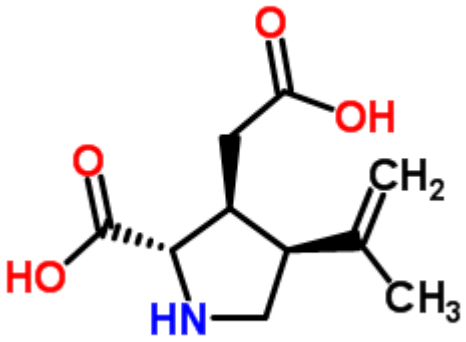
Figure 1. Chemical structure of kainic acid.

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